REMARKS

Claims 1-46 were pending prior to this response. By the present communication, claims 3, 10-12, 28 and 44 have been cancelled, and claims 1, 2, 6, 7, 13, 14, 16-19, 21, 23, 24, 29, 34, 39, and 43 have been amended to define Applicants' invention with greater particularity. The amendments add no new matter, being fully supported by the Specification and originally filed claims. Accordingly, claims 1, 2, 4-9, 13-27, 29-43, 45, and 46 are currently pending.

Priority

The Office Action indicates that claim 1-16 drawn to enhancing capacity of impaired bone marrow cells is granted priority to the filing date of the instant application, but then states that claims 1-17 are rejectable over Iwaguro et al. (Feb. 2002). It is assumed for the purposes of this response that, due to the contradiction in stating which claims are involved, the Examiner meant to say that claims 1-16, not 1-17, are rejectable over Iwaguro et al.

The Rejection under 35 U.S.C. § 112, Second Paragraph

Applicants respectfully traverse the rejection of claims 1 and 17 for indefiniteness based on alleged ambiguity of the phrase "derived from" as used therein because the nature or number of steps required to obtain a derivative are unknown. For clarity, claim 1 has been amended to delete the phrase "derived from" and now recites: "thereby enhancing capacity of the bone marrow cells and/or the conditioned medium to promote development of collateral blood vessels in the patient . . ." Similarly, claim 17 has been amended to delete the phrase "derived from" and now recites" "directly administering to a desired site in the patient an effective amount of the transfected early attaching cells and/or the conditioned medium to produce collateral blood vessel formation at the site in the patient." Applicants submit that the amendments to claims 1 and 17 obviate the grounds for the indefiniteness rejection as to this point.

With regard to claim 14, the Examiner asserts that the term "stimulating" is indefinite as used therein because the term allegedly lacks specific definition in the Specification. To obviate

the rejection, claim 14 has been amended to require that the cells are stimulated "by contact with HIF-1 or EPAS-1 or by exposure to hypoxia." Support for the amendment to claim 14 is found in paragraphs [0030] and [0031], among others.

In view of these amendments and the above remarks, Applicants submit that the pending claims meet all requirements under 35 U.S.C. § 112, second paragraph, and reconsideration and withdrawal of the rejection are respectfully requested.

The Rejection Under 35 U.S.C. § 112, First Paragraph

Applicants respectfully traverse the rejection of claims 1-46 under 35 U.S.C. § 112, first paragraph, for allegedly lacking an enabling disclosure commensurate with the scope of the claims. Applicants disagree with the Examiner's assertion that the Specification provides enablement only for claims directed to treatment of hind-limb ischemia in mice.

First, it should be noted that claims 1-16 and 39-46 do not pertain to a method of treatment of any subject, whether humans or of mice. Rather, claim 1 recites "a method of enhancing capacity of impaired bone marrow to promote development of collateral blood vessels". Thus, claims 1-16 pertain to methods for "treating" bone marrow cells so as to enhance their ability to secrete angiogenic cytokines when the donor has a disorder, such as aging, that impairs the ability of the bone marrow cells as compared with healthy, young cells. Claim 39 recites "A therapeutic composition comprising early attaching cells derived from bone marrow . . . " Thus, claims 39-46 are composition claims. The Examiner does not allege that the Specification fails to teach those of skill in the art how to utilize bone marrow to prepare the therapeutic composition used in the method claims, claims 17-38.

With regard to the method claims, the Examiner asserts that the claims constitute "gene therapy" and hence automatically must be rejected over the Verma and Anderson references published regarding the state of gene therapy prior to the 1997-98 era. These articles obviously do not reflect the state of gene therapy in the present times.

In response to the Examiner's assertions regarding the unpredictability of gene therapy, Applicants submit that the present invention is based on studies showing that, in one embodiment, reinjection of the stimulated bone marrow, including any products produced by the bone marrow after stimulation, provides an environment necessary for arteriogenesis in vivo, such as development of collateral blood supply to ischemic tissue. Moreover, in one embodiment of the invention, Applicants have discovered that conditioned medium produced by culture of cells produced by growing bone marrow cells is effective alone to promote development of collateral blood flow in ischemic tissue of heart or limb. Thus, Applicants have discovered that (in embodiments of the invention not covered by the present claims) the process involved in the invention method steps leads to successful transplant *and* subsequent growth of collaterals, irrespective of whether the cultured bone marrow cells have been transfected with a gene that encodes a particular transcription factor or cytokine, such as HIF-1 or EPAS1, MCP-1 GM-CSF, PR39, and the like, because the cultured bone marrow cells themselves endogenously produce a battery of angiogenic cytokines.

In other words, in the invention methods, expression of the transfected genes in the cells, with resultant increase in expression within the cell of multiple endogenous angiogenic gene products, and secretion of such products into the culture medium, is sufficient to *augment* the development of collateral blood vessels in ischemic tissue of the patient in heart or peripheral limb. It is not a requirement that the transfected genes continue to produce the cytokines for prolonged periods after implantation into the affected area, although transient expression of the transgenes after implantation is believed to contribute to the therapeutic effect. Thus, Applicants submit that the stock reaction to the term "gene therapy" used as the basis of the rejection for alleged non-enablement of the claims is not fitting in the present case.

Since the filing of this application, Applicants have further tested the invention methods and compositions in laboratory studies to prove its validity. Attached are copies of three publications by the inventors, one manuscript, one article that is in review angiopgenic cytokine(s) or whose protein product stimulates the expression of other angiogenic genes. Taken

together these documents corroborate the teachings of the Specification, and further illustrate the validity of the claimed methods and compositions.

Thus, Applicants disagree with the Examiner's assertion that the disclosure is enabling for direct administration of the invention compositions to ischemic hind limb of mice, but not for administration to heart, especially in human heart. Applicants teach that the invention methods for enhancing collateral blood vessel formation are equally effective when a composition comprising an effective amount of either the transfected early attaching cells or the conditioned medium produced by allowing expression of the transgene during culture is administered to heart or limb tissue, for enhancing development of collateral flow. Like administration to hind limb, direct administration to the heart involves intramuscular injection into heart tissue. Applicants submit that the Examiner has failed to present a rational basis for the conclusion underlying the rejection that the invention methods and compositions would stimulate growth of new blood vessels or remodeling of *existing* blood vessels that are in the treated tissue, but are too small to result in substantial flow, in peripheral limb tissue (i.e., hind limb of mouse) but would not have a similar effect on blood vessels in heart ischemic tissue..

Since the filing of the present application, studies have been published showing the restoration of blood flow in myocardial tissue of porcine models and in human patients with advanced coronary artery disease upon administration of autologous bone marrow stromal cells (See Al-Khaldi A, et al., *Ann Thorac Surg.* 2003. 75:204-209, a copy of which is attached) or conditioned medium containing bone marrow-derived angiogenic growth factors such as VEGF and MCP-1 (See S Fuchs, et al., 2003. JACC, Vol. 41(10)1721-4, a copy of which is attached). There is absolutely no basis presented by the Examiner for supporting the assertion that when such cells are transfected by a transgene that expresses additional angiogenic cytokine(s) or whose protein product stimulates the expression of other angiogenic genes, implantation of the cells (i.e., with or without conditioned medium that captures the expressed endogenous and transgenic cytokines) will be any less effective.

In addition, the post-filing article by co-applicants S. Fuchs and S.E. Epstein describes studies in which cultured marrow-derived stromal cells with conditioned medium *or*

conditioned medium alone, when obtained by culturing marrow-derived stromal cells under either normoxic or hypoxic conditions, enhance collateral flow recovery and remodeling when injected into murine hind limb ischemic tissue (T. Kinnaird et al. 2004. *Circulation Research* March 19, 2004:678-685, a copy of which is attached). Applicants have elsewhere demonstrated that such stromal cells are obtained by culturing bone marrow, autologous or non-autologous (T.D. Kinnaird et al. "Local delivery of marrow-derived stromal cells augments collateral perfusion through paracrine mechanisms." *Circulation*, 2004;109:1543-1549 a copy of which is attached)). ELISA analysis of the conditioned medium so produced was shown to comprise a complex variety of endogenously expressed cytokines, with the marrow-derived stromal cells acting as tiny cytokine factories (See table, page 680).

This study additionally showed that the bone marrow derived cells need not be incorporated into vessel structures to accomplish the therapeutic result. In fact, the conditioned medium alone was sufficient to promote development of collateral vessels and reverse the effect of limb damage by a mechanism described as paracrine signaling (Col 1, page 684).

Above all, it should be noted that this invention does not rely on transdifferentiation of the injected bone marrow cells into cells of the tissue into which they are injected or upon continued expression of transgenes after cell implant, but relies instead upon survival of the bone marrow cells and expression of transgenes long enough (i.e., transient survival) to secrete into the tissue (if cells are injected) cytokines (including, but by no means limited to transgeneic cytokines or cytokines whose production is stimulated by the products of the transgenic genes) that stimulate the formation of new blood vessels in vivo or result in the expansion of existing, but very small, blood vessels. Similar considerations apply to the direct injection of the cytokines contained in the conditioned medium. to secrete into the tissue (if cells are injected) cytokines (including, but by no means limited to transgeneic cytokines or cytokines whose production is stimulated by the products of the transgenic genes) that stimulate the formation of new blood vessels in vivo or result in the expansion of existing, but very

small, blood vessels. Similar considerations apply to the direct injection of the cytokines contained in the conditioned medium.

Thus, Applicants submit that those of skill in the art are provided all the guidelines required to make and use the invention methods, as defined by the present claims, for enhancing collateral blood vessel formation in heart *or limb tissue* experiencing impaired blood flow without undue experimentation. Accordingly, reconsideration and withdrawal of the rejection for alleged lack of enablement are respectfully requested.

The Rejection under 35 U.S.C. § 112, First Paragraph

Applicants respectfully traverse the rejection of claims 1-9, 11-12, 14-16, 18-28, and 33-46 for lacking a written description sufficient to convey to those of skill in the art that the inventors were in possession of the invention as claimed at the filing of the present application. In particular, the Examiner observes that the rejected claims encompass an "enormous breadth of the growth and angiogenic factors" and that a biotechnological invention cannot necessarily claim a genus after only describing a limited number of species because there may be unpredictability in the results obtained from other species (Office Action, page 8) However, Applicants submit that more than a "limited number" of representative examples of the genus has been described in the Specification. As the Examiner acknowledges, five FGFs NOS and PR39, as well as GM-CSF, MCP-1, EPAS1 and HIF-1α have been specifically described, a total of 11 angiogenic cytokines.

However, to advance prosecution and reduce the issues, the limitations of claim 10 have been introduced into claims 1, 17, and 39. As claims containing these limitations, such as original claim 10, were not included in the written description rejection, Applicants submit that all claims now meet the written description requirements under 35 U.S.C. § 112, first paragraph. Accordingly, reconsideration and withdrawal of the rejection are respectfully required.

The Double Patenting Rejection

Applicants respectfully traverse the provisional rejection of claims 17-28 and 31-46 under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 14-18, 9, 24, 25, 27 and 30-31 of copending Application No. 09/868,411. Submitted herewith is a Terminal Disclaimer disclaiming the terminal part of any patent granted on the subject matter of the above-identified U.S. Patent Application Serial No. 10/618,183, filed July 10, 2003, that would extend beyond the expiration date of any patent that may be granted based on U. S. Patent Application No. 09/868,411 and stating that any patent so granted on this application shall be enforceable only for and during such period that the legal title to the subject matter of said patent shall be the same as the legal title to U. S. Patent Application No. 10/618,183. In view of the Terminal Disclaimer submitted herewith, Applicants submit that copending Application No. 09/868,411 is no longer available as a reference against the present application. Accordingly, reconsideration and withdrawal of the provisional rejection of claims 17-28 and 31-46 under the judicially created doctrine of obviousness-type double patenting are respectfully requested.

The Rejection under 35 U.S.C. § 103

Applicants respectfully traverse the rejection of claims 1-7 and 11-12 under 35 U.S.C. § 103 over Iwaguro et al., (Circulation 105(6):732-38 (Feb. 12, 2002); hereinafter "Iwaguro"). Applicants submit that the invention method for enhancing capacity of bone marrow to promote development of collateral blood vessels in a patient having a condition that impairs naturally occurring angiogenic processes as compared with that found in normal young healthy individuals, as defined by claim 1, distinguishes over the disclosure of Iwaguro by requiring:

"growing bone marrow cells under suitable culture conditions in a suitable growth medium for a period of time sufficient to promote production by the bone marrow cells of early attaching cells;

transfecting at least a portion of the early attaching cells with an adenovirus vector comprising a polynucleotide that encodes one or more agents selected from hypoxia inducing

factor-1 (HIF-1), endothelial PAS domain protein 1 (EPAS1), Monocyte Chemoattractant Protein 1 (MCP-1), granulocyte-monocyte colony stimulatory factor (GM-CSF), PR39, a fibroblast growth factor (FGF), and a nitric oxide synthase (NOS), and

culturing the transfected early attaching cells in a culture medium to produce the one or more agents and conditioned medium,

thereby enhancing capacity of the bone marrow cells and/or the conditioned medium to promote development of collateral blood vessels in the patient into which the cells and/or the conditioned medium are delivered as compared with that of either non-transfected cells or conditioned medium similarly obtained using non-transfected early attaching cells."

Iwaguro is absolutely silent regarding "bone marrow cells" or "bone marrow early attaching cells". Ewaguro is also silent regarding the effect of aging and diseases such as hypercholesterolemia on the function of bone marrow cells in any type of bone marrow transplant method. In addition, Iwaguro is absolutely silent regarding use of conditioned medium from early attaching cells obtained by growing bone marrow cells as a therapeutic composition.

Instead, Iwaguro's method describes collection of *mononuclear cells from peripheral blood* of human volunteers, expansion on culture dishes, and transfer of genes encoding VEGF-A into endothelial progenitor cells (EPCs) during ex vivo expansion as a potential means for overcoming liabilities in EPC function brought on by aging, hypercholesterolemia, and the like. Conditioned medium from EPCs not transfected with the growth factor was used as a control in the Iwaguro experiment without any suggestion that the conditioned medium from transfected cells would serve as a substitute for the transfected EPCs.

In addition, Iwaguro's experiment is designed to track "angiogenesis" but not "arteriogenesis". Capillary density and perfusion in treated mouse hindlimb was observed, but Iwaguro fails to suggest development of collateral arteries by the disclosed technique.

Moreover, Applicants submit that those of skill in the art would not be motivated by Iwaguro to substitute progenitor cells obtained from bone marrow, such as mesenchymal cells or stromal progenitor cells for EPCs with any reasonable degree of expectation of success. In fact,

Iwaguro's conclusions would dissuade those from skill in the art from trying such an experiment. Iwaguro explicitly advises on this point:

> Testing such alternative approaches [using non-EPC circulating cells], however, is currently precluded by the lack of non-EPC cells that can (a) be ex vivo-transduced with equal efficiency; (b) circulate in vivo for some reasonable time period; and (c) be recruited to as well as incorporate into foci of neovascularization.

(Iwaguro, page 737, 2nd col.). Indeed, it is the discovery of the Applicants that the bone marrow-derived cells produced by in vitro expansion (i.e., early attaching cells) can be transfected with high efficiency and do not need to be incorporated large scale into developing neovascularization to stimulate formation of collateral blood flow in ischemic tissue. Applicants have discovered that, when injected into ischemic tissue, the endogenous and transgenic cytokines secreted by the cells (or simply condition medium containing such cytokines secreted from the cells, even when not transfected with genes that express angiogenesis promoting cytokines) stimulate the growth of new blood vessels or remodeling of *existing* blood vessels that are in the treated tissue, but are too small to result in substantial flow.

Thus, Iwaguro's comments actually lead away from the invention methods and compositions. For this reason the cited art would dissuade, rather than motivate, those of skill in the art to adapt Iwaguro's methods along the lines of the present invention and would certainly not provide a reasonable expectation of success if such an attempt were to be contemplated or carried out.

Accordingly, Applicants submit that *prima facie* obviousness of the invention methods for enhancing capacity of bone marrow to promote development of collateral blood vessels in a patient having a condition that impairs naturally occurring angiogenic processes as compared with that found in normal young healthy individuals is not established over Iwaguro under 35 U.S.C. § 103(a). Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

In view of the above amendments and remarks, Applicants request favorable action on all pending claims. If the Examiner would like to discuss any of the issues raised in the Office Action, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

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Attachments:
1. Al-Khaldi et al., "Therapeutic angiogenesis using autologous bone marrow stromal cells: improved blood flow in a chronic limb ischemia model", *Ann Thorac Surg.* 2003 Jan; 75(1):204-9
2. Fuchs et al., "Catheter-based autologous bone marrow myocardial injection in no-option patients with advanced coronary artery disease: a feasibility study", *J Am Coll Cardiol.* 2003 May; 41(10):1721-4
3. Kinnaird et al, "Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms", *Circ Res.* 2004 Mar 19;94(5):678-85
4. Kinnaird et al., "Local Delivery of Marrow-Derived Stormal Cells Augments Collateral Perfusion Through Paracrine Mechanisms", *Circulation.* 2004 Mar 30;109(12):1543-9

Enclosure: Terminal Disclaimer